The Beryllium Lymphocyte Proliferation Test (BeLPT)

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Recognition of Beryllium Health Effects

1940s: Reports of adverse health effects associated with beryllium exposure.

- Acute disease with high exposures.
- Chronic disease present.

1950s: Be exposure standard (2 $\mu g/m^3$) implemented but cases of CBD continue to be reported

Recognition of Beryllium Health Effects

1980s: New test (BeLPT) identifies beryllium sensitization, a precursor to CBD.

2000: Department of Energy mandates CBD protection programs

2001: EEOICPA: Compensates employees injured during employment in the nuclear weapons development effort

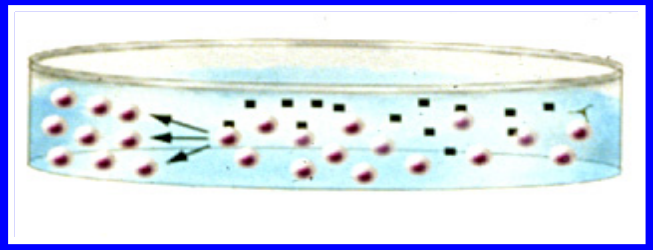
Extent Of Beryllium Use

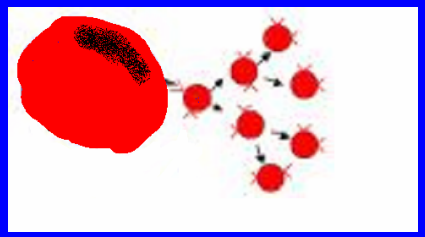
- NIOSH estimates
  - At risk: 134,000 in the US
    (J Occup Environ Hyg 2004 1:648)
  - Probably conservative- Doesn't include "downstream users"
- Study of Construction Trades Workers at 3 DOE sites
  - Estimate 230,000 exposed (Welch 2004)

Importance of an Accurate Diagnosis

- Intervene early
  - If CW, remove/reduce exposure
  - Reduce exposures to workers in same job
- If CBD
  - Follow closely for progression of symptoms
  - Advise to maintain health (exercise, preventive vaccines)
  - Treat to preserve lung function

Beryllium Lymphocyte Proliferation Test (BeLPT)



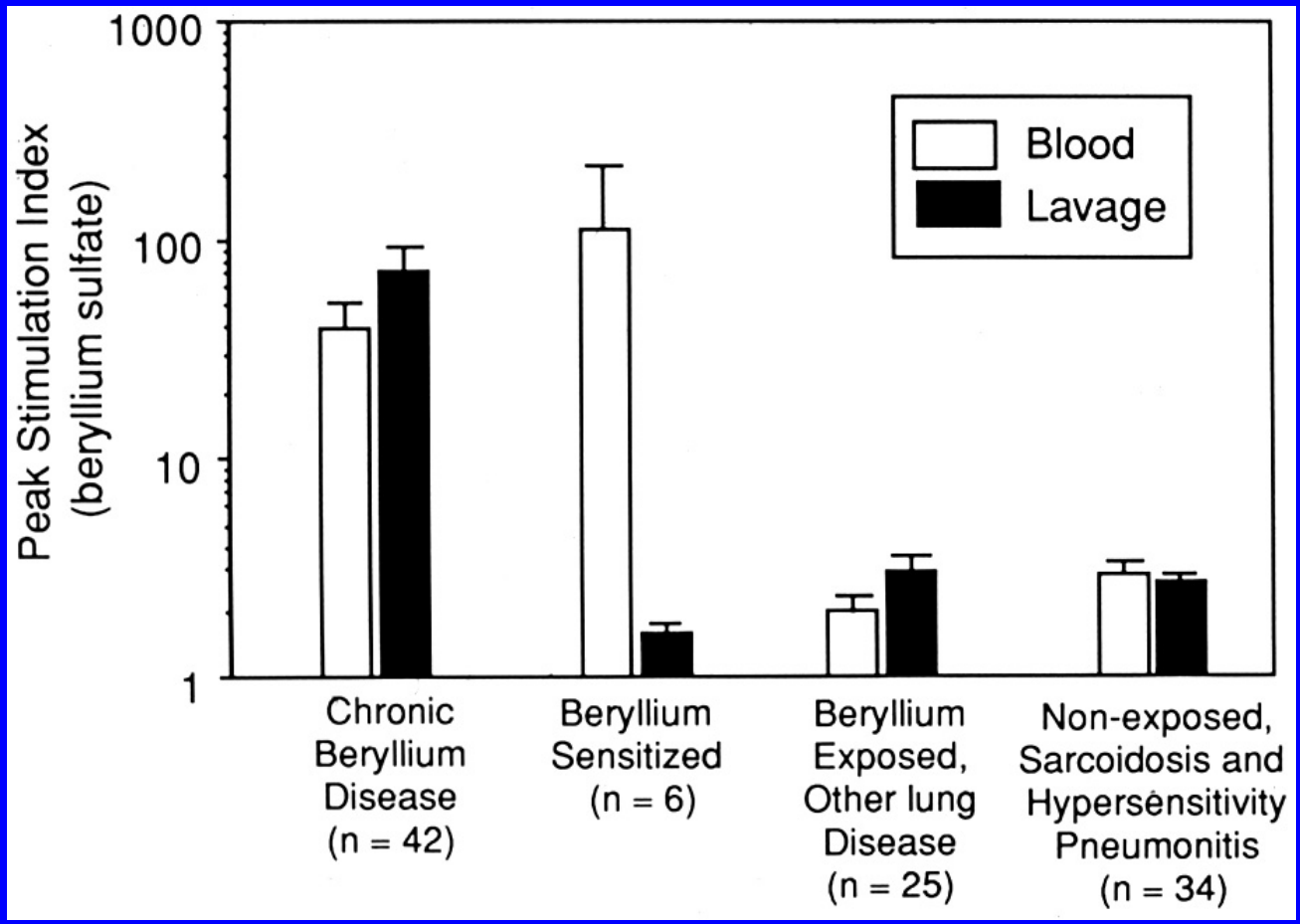


- Measures blood cells response to beryllium
- Identifies beryllium sensitization
- Further clinical testing needed to diagnose CBD

BeLPT Characteristics

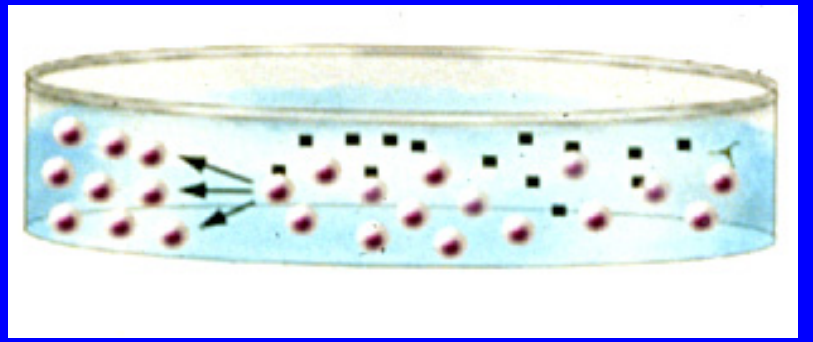
- Detects a beryllium-specific cell mediated immune response Identify CBD at early stages
- Differentiates CBD from lung disease of other etiology (sarcoidosis, COPD, HP)
- Increased sensitivity and specificity to diagnose BeS/CBD compared with other tests
  - Occupational History
  - CXR or CT scan
  - Spirometry

The BeLPT is Specific to Be - Related Health Effects



How is the Test Performed

- Culture peripheral blood or BAL cells with and without beryllium
- Measure incorporation of ^{3}H-Td for proliferation
- Stimulation index or SI : ratio of Be-stimulated to unstimulated cpm
- Use positive controls to ensure cells are able to proliferate



Lymphocyte Proliferation Test: Peripheral Blood

NORMAL LPT

Patient Name:
ID Number: OS#:
Date of Test: Accession#:
Referring Physician:
Technician:

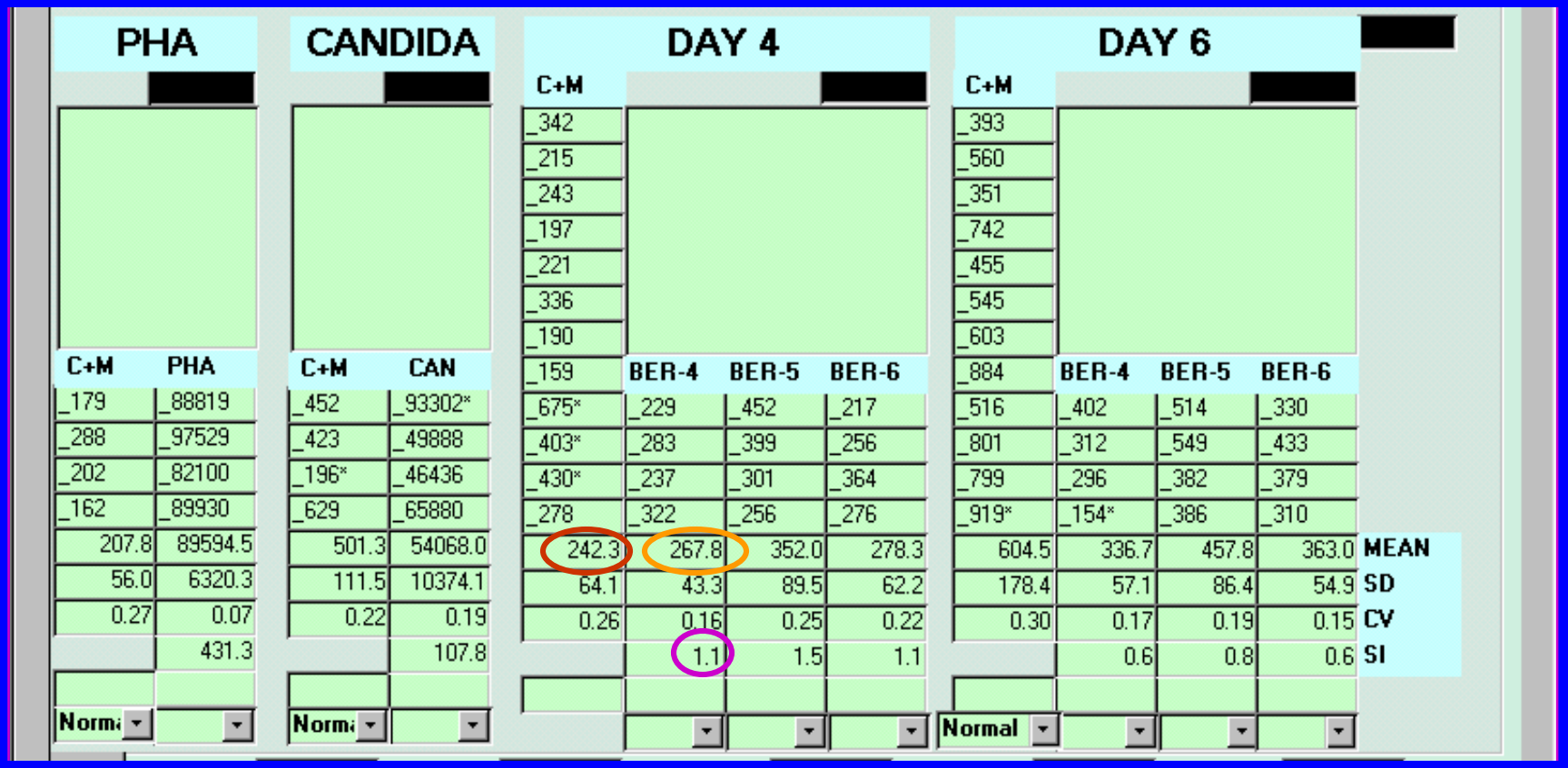
Results	Mean Stimulation Index		
Mitogens:	Day 3	Day 4	Day 6
Phytohemagglutinin	431.3		
Antigens:			
Candida			107.8
Beryllium Sulfate:			
1X10-4 M		1.1	0.6
1X10-5 M		1.5	0.8
1X10-6 M		1.1	0.6

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Normal lymphocyte proliferation to beryllium sulfate.

Note: A normal blood result does not exclude the diagnosis of Chronic Beryllium Disease. If the clinical setting is compatible, a beryllium lymphocyte proliferation test using bronchoalveolar lavage lymphocytes is recommended.

A normal result is all values equal or below the cutoff value of 2.5.

Raw Data for Normal Blood LPT



PHA		CANDIDA		DAY 4				DAY 6				
C+M	PHA	C+M	CAN	C+M				C+M				
_179	_88819	_452	_93302*	_342				_393				
_288	_97529	_423	_49888	_215				_560				
_202	_82100	_196*	_46436	_243				_351				
_162	_89930	_629	_65880	_197				_742				
207.8	89594.5	501.3	54068.0	_221				_455				
56.0	6320.3	111.5	10374.1	_336				_545				
0.27	0.07	0.22	0.19	_190				_603				
	431.3		107.8	_159	BER-4	BER-5	BER-6	_884	BER-4	BER-5	BER-6	
				_675*	_229	_452	_217	_516	_402	_514	_330	
				_403*	_283	_399	_256	_801	_312	_549	_433	
				_430*	_237	_301	_364	_799	_296	_382	_379	
				_278	_322	_256	_276	_919*	_154*	_386	_310	
Norma		Norma		242.3	267.8	352.0	278.3	604.5	336.7	457.8	363.0	MEAN
				64.1	43.3	89.5	62.2	178.4	57.1	86.4	54.9	SD
				0.26	0.16	0.25	0.22	0.30	0.17	0.19	0.15	CV
					1.1	1.5	1.1		0.6	0.8	0.6	SI
								Normal				

Calculate Stimulation Index (D4B4) 267.8 / 242.3 = 1.1

Lymphocyte Proliferation Test: Peripheral Blood

ABNORMAL LPT

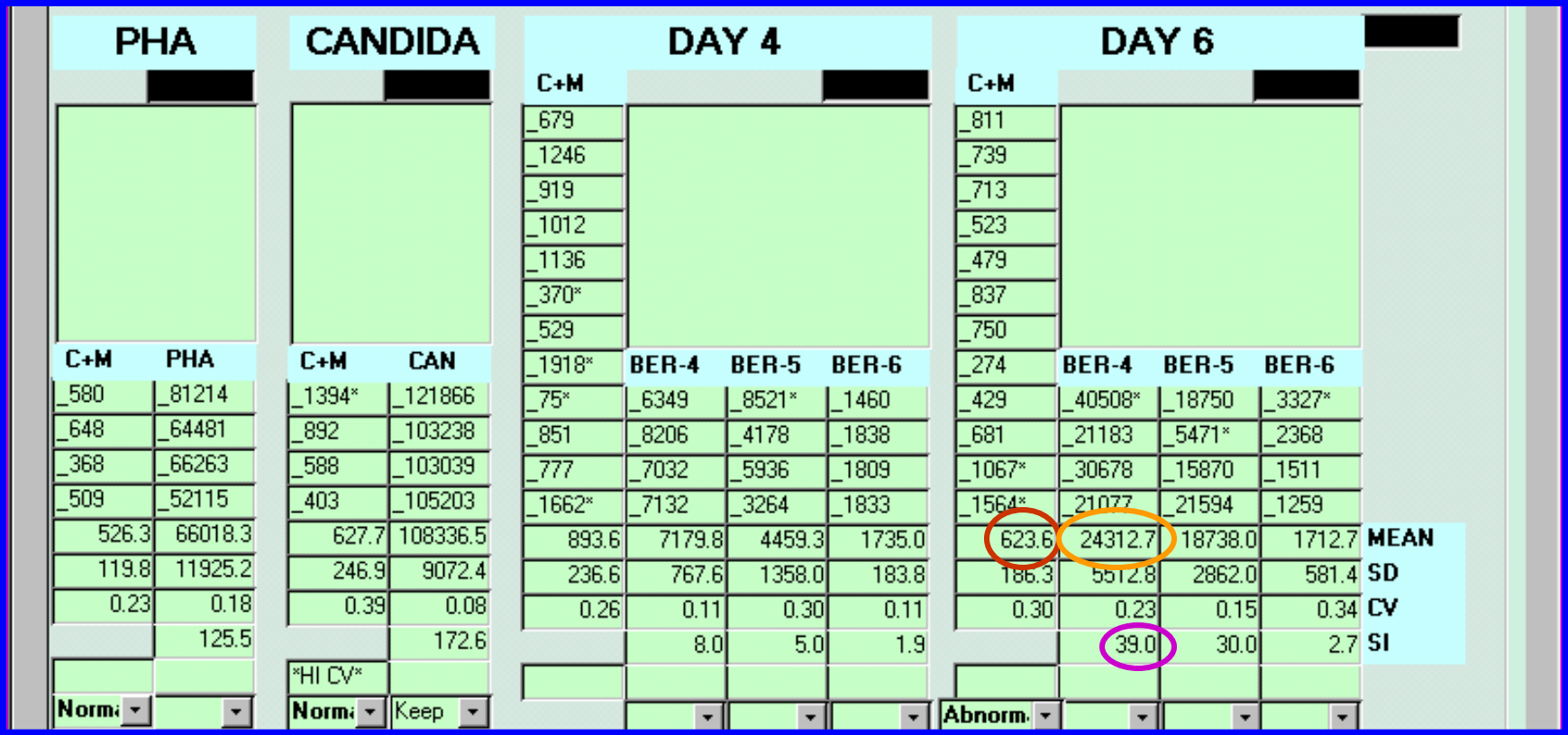
Patient Name:
ID Number: OS#:
Date of Test: Accession#:
Referring Physician:
Technician:

Results	Mean Stimulation Index		
Mitogens:	Day 3	Day 4	Day 6
Phytohemagglutinin	125.5		
Antigens:			
Candida			172.6
Beryllium Sulfate:			
1X10-4 M		8.0	39.0
1X10-5 M		5.0	30.0
1X10-6 M		1.9	2.7

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Abnormal lymphocyte proliferation to beryllium sulfate.

Note: An abnormal result is two or more values above the cutoff value of 2.5.

Raw Data for Abnormal LPT



PHA		CANDIDA		DAY 4				DAY 6				
				C+M				C+M				
				_679				_811				
				_1246				_739				
				_919				_713				
				_1012				_523				
				_1136				_479				
				_370*				_837				
				_529				_750				
C+M	PHA	C+M	CAN	_1918*	BER-4	BER-5	BER-6	_274	BER-4	BER-5	BER-6	
_580	_81214	_1394*	_121866	_75*	_6349	_8521*	_1460	_429	_40508*	_18750	_3327*	
_648	_64481	_892	_103238	_851	_8206	_4178	_1838	_681	_21183	_5471*	_2368	
_368	_66263	_588	_103039	_777	_7032	_5936	_1809	_1067*	_30678	_15870	_1511	
_509	_52115	_403	_105203	_1662*	_7132	_3264	_1833	_1564*	_21077	_21594	_1259	
526.3	66018.3	627.7	108336.5	893.6	7179.8	4459.3	1735.0	623.6	24312.7	18738.0	1712.7	MEAN
119.8	11925.2	246.9	9072.4	236.6	767.6	1358.0	183.8	186.3	5512.8	2862.0	581.4	SD
0.23	0.18	0.39	0.08	0.26	0.11	0.30	0.11	0.30	0.23	0.15	0.34	CV
	125.5		172.6		8.0	5.0	1.9		39.0	30.0	2.7	SI
		HI CV										
Norma		Norma	Keep					Abnorm				

Calculate Stimulation Index (D6B4) 24312.7 / 623.6 = 39.0

Lymphocyte Proliferation Test: Peripheral Blood

UNINTERPRETABLE

Patient Name:
ID Number: OS#:
Date of Test: Accession#:
Referring Physician:
Technician:

Results	Mean Stimulation Index		
Mitogens:	Day 3	Day 4	Day 6
Phytohemagglutinin	36.0		
Antigens:			
Candida			4.0
Beryllium Sulfate:			
1X10-4 M		---	---
1X10-5 M		---	---
1X10-6 M		---	---

Normal response to mitogen.
Normal response to antigen. (Normals: >3.0)
Uninterpretable lymphocyte transformation to beryllium sulfate.

Why is a Test Uninterpretable

- Cells “over” respond in all conditions, possibly masking a true lymphocyte proliferation in Be stimulated condition(s)
- Positive controls not positive
- Lab error

Specificity and Sensitivity of the BeLPT

Maier AOEH, 2001

Study	Individuals Enrolled, n	BeS/CBD, n (%)	Estimated Sensitivity	Estimated Specificity	Positive Predictive Value
Mroz et al, JACI 1991	35	17 (49%)	94%	100%	
Stokes et al, JOEM 1991	57	27 (47%)	38%	97%	
Kreiss et al, JOEM 1993[+]*	505	9 (2%)	89%	NA	100%
Kreiss et al, ARRD 1993[+]*	895	18 (2%)	100%	NA	100%
Stange et al, Environ Health Perspect 1996[+]*	4268	101 (2%)	97%	NA	

Predictive Value of Clinical Tests in the Diagnosis of CBD

Kreiss et al JOEM 1989

Test	Positive Predictive Value	Negative Predictive Value
Symptoms	1.6-10.5%	98.3-99%
FEV_1	2.9%	98.3%
FVC	3.3%	98.2%
FEV_1/FVC	3.4%	98.5%
Chest Xray	12.9%	98.9%
LPT	100%	99.8%

Alternative Screening Tests for CBD

- Chest X-ray
  - PV+ = 12.9% (Kreiss et al, 1993)
  - PV+ = 0.8% (Stange et al, 1996)
- Spirometry
  - PV+ of FEV_1 = 2.9% (Kreiss et al, 1993)
  - PV+ of FVC = 3.3% (Kreiss et al, 1993)
- Symptoms
  - PV+ of COUGH = 3.2% (Kreiss et al, 1993)
  - PV+ of DYSPNEA = 5.0% (Kreiss et al, 1993)

Interlaboratory Agreement

- Previous report 85-96% between labs, 30-60% for abnormal tests (Stange, Env Health Perspec 1996)
- Prelim evaluation split test from 2 labs (n=825)
  - 716/825 agreed (87%)
  - 703/716 negative (98% of agreed, 85% of total)
  - Total positive: 44 (5%)

BeLPT compared to other medical screening tests

	Test	Sensitivity	Specificity	Follow-up test
Breast CA	Mammography	75 – 88%	83 – 99%	Biopsy
Colon CA	Fecal Occult Blood	26 – 92%	90 – 99%	Colonoscopy
Neural Tube Defects	AFP	56 – 91%	5 – 10%	Amniocentesis
Prostate CA	PSA	29 – 80%	75%	Biopsy
BeS/CBD	BeLPT*	74 – 91%	96 –99%	Bronchoalveolar lavage/biopsy

Considerations in using the BeLPT

- Borderline/uninterpretable
- False negative
- False positives
- Lack of concordance between some labs
- At least 7 days for results
- Does not discriminate between BeS and CBD

Efforts to Standardize Testing

- CABST
- ICPT
- DOE Specification, April 2001 (update expected)
  - Minor differences remain:
    - Days of culture
    - Cut-off value determination
    - Serum lots

What if There are Problems with the BeLPT?

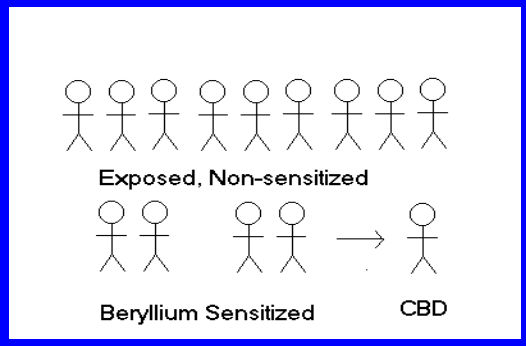
- Sometimes the cells fail to respond to beryllium. A negative BeLPT does not exclude the diagnosis of CBD
  - More common in smokers
- Beryllium skin patch testing can be used to demonstrate an immune response to beryllium

Benefits of the BeLPT

- Redefined and broadened our understanding of beryllium related health effects:
- Can detect health effects at an early stage
- Higher sensitivity and specificity than other screening tests
- Can be used in screening and surveillance mode to help evaluate processes/tasks

Applications of the BeLPT

- Diagnosis of beryllium related health effects
  - Beryllium Sensitization
  - CBD



- Workplace screening and surveillance
  - Prevalence of health effects
  - Work practices and job titles with increased risk

Summary

- BeLPT provides non-invasive test to screen for Be-health effects
  - Specific and sensitive
  - Used in evaluation of process/exposure risks
- Studies needed of other tests
  - Discriminate BeS and CBD
  - Enhance performance of BeLPT
  - Increased specificity/sensitivity

Questions ?

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